

1.4 DNA Extraction and Purification in Sample

- 1.4.1** Grind about 0.5 g of plant tissues with liquid nitrogen, and transfer the powder to a 2 mL centrifuge tube.
- 1.4.2** Add 0.5 mL of extract A, mix, and put in a 65 °C water bath for 1 hour.
- 1.4.3** After bathing, add 1 mL of extract B: extract C=1:1 mixture to the tube, mix for 30 seconds, and then centrifuge at 12,000 rpm for 5 minutes.
- 1.4.4** Pipette the upper aqueous phase to a new 2 mL centrifuge tube, add double volume of the pre-cooled anhydrous alcohol, 10 % volume of settling agent 1 and 4 µL of settling agent 2, mix, and allow to settle at -20 °C for 1 hour.
- 1.4.5** After settlement, centrifuge at 12,000 rpm at 4 °C for 15 minutes, and pour away the supernatant carefully. At this point, white sediment can be seen at the bottom of the EP tube.
- 1.4.6** Add 1 mL of the pre-cooled washing solution, flip the EP tube to mix, centrifuge at 12,000 rpm at 4 °C for 5 minutes, discard the supernatant, and invert the EP tube onto a filter paper for drying.
- 1.4.7** Add 30 µL of dissolving solution to the dried EP tube for sediment dissolution, and maintain the resultant solution at -20 °C.

1.5 PCR Reaction System

See Tables 2 and 3 for the PCR reaction reagents and conditions.

Table 2 PCR Reaction Reagents

	Consumption	Final concentration
SYBR [®] Premix Ex Taq II (Tli RNaseH Plus) (2')	10.0 mL	1'
PCR Forward Primer (10 mM)	0.8 mL	0.4 mM
PCR Reverse Primer (10 mM)	0.8 mL	0.4 mM
DNA template	2.0 mL	20 ng/mL
dH ₂ O (sterilized distilled water)	6.4 mL	
Total volume	20.0 mL	

Table 3 PCR Reaction Parameters

Action	Time/s	Temperature/°C
Activation and pre-denaturalization of DNA active enzyme	30	95
PCR (45 cycles)		
Denaturalization	30	95
Annealing	30	55
Extension	60	72
Post-cycle retention	180	72

1.6 MultiNA Determination

The PCR amplified products were subject to MultiNA determination. Based on the theoretical size of the fragment, the 500 bp reagent kit was selected for determination in the experiment. In order to verify the accuracy of measurement, this paper also includes a negative control experiment, where the DNA template was not used.

Discussion of Results

Fig. 1 and Fig. 2 are the gel diagram and electropherogram of MultiNA determination of genetically modified 356043 soybean, respectively. The experimental results indicate that the fragment length of the soybean endogenous Lectin gene was 166 bp, largely consistent with the theoretical fragment length of 162 bp, indicating that the soybean genome has been extracted successfully and the PCR process has been implemented successfully. For the 356043 transgene, the results indicate that a 153 bp gene fragment was detected, largely consistent with the theoretical fragment length of 145 bp, indicating that the 356043 transgene has been detected successfully. No relevant fragment was detected in the negative control experiment, indicating no false positive detection.

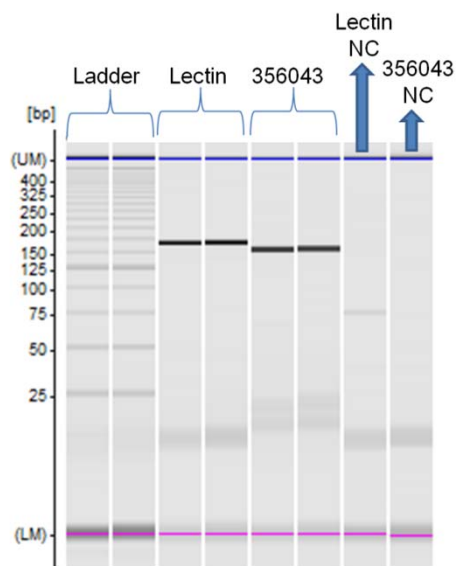


Fig. 1 Gel Diagram of MultiNA Determination of Genetically Modified 356043 Soybean (NC: negative control)

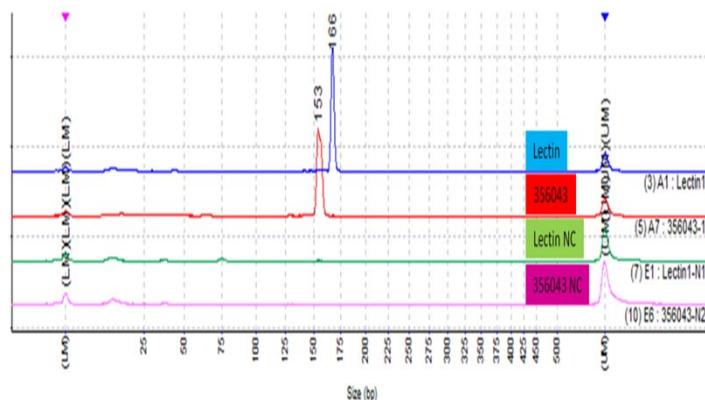


Fig. 2 Electropherogram of MultiNA Determination of Genetically Modified 356043 Soybean (NC: negative control)

Conclusion

This paper establishes a method for the qualitative determination of genetically modified 356043 soybean using the Shimadzu MCE-202 MultiNA based on molecular biological technology. This method is sensitive, easy to operate and accurate for the determination of the 356043 variety of genetically modified soybean.